**Speaker:** Tom Sadler, Ph.D.

**Topic:** Overview of Cardiovascular Embryology and Teratogenesis

**Objectives:**

1) Describe the normal development of the fetal cardiovascular system.
2) Identify the stages of pregnancy in which major developmental milestones occur in the cardiovascular system.
3) Name the most prevalent types of birth defects occurring in the cardiovascular system.
4) Name three birth defect syndromes which commonly involve heart defects.

**Outline:**

1) Critical weeks of cardiac development
2) Origin of tissues for:
   a) Atrial septum
   b) Ventricular septum
   c) Atrioventricular canals
   d) Conotruncal septum
3) Role of neural crest cells in heart and face development
4) Role of endocardial cushion tissue in most cardiac defects
6) Origins of common cardiac defects:
   a) ASD-atrial septal defects
   b) VSD-ventricular septal defect
   c) Transposition of the great vessels
   d) Pulmonary stenosis
   e) Tetralogy of Fallot

**Abstract:** Heart development begins when endothelial cells from mesoderm coalesce to form a horseshoe-shaped tube cranial to the developing neural plate. As the embryo folds ventrally, the sides of this tube are brought together and fuse to form a single channel. The caudal end receives venous blood, while the cranial end sprouts vessels, including the aorta and pulmonary arteries, to become the arterial outflow tract. As the tube forms, it expands and folds (loops) on itself. Expansion creates the primitive chambers and looping initiates separation of these chambers into atria and ventricles. Completion of septation is accomplished by expansion of the endothelial lining on the inner surface of the tube to form endocardial cushions. Two of these cushions, located on either side of the atrioventricular opening, grow toward the midline and fuse to separate this opening into two parts: the mitral opening, between the left atrium and ventricle; and the tricuspid opening, between the right atrium and ventricle. In addition, a septum grows downward from the roof of the atrium, contacts the atrioventricular endocardial cushions, and separates the left and right atria. Similarly, a septum grows upward from the ventricles toward the atrioventricular region fuses with the cushions, and separates the left and right ventricles. Finally, the outflow tract is separated into the aorta and pulmonary vessels by growth of endocardial cushion tissue from the walls of the original channel toward the midline, where they eventually fuse. Formation of the heart is one of the most complex embryological events and many processes can, potentially, be disrupted genetically or by environmental factors. Thus, heart defects, such as ventricular and atrial septal defects, tetralogy of Fallot, pulmonary stenosis, etc. are common. The origins of these defects can be better appreciated if one understands the normal embryology.

**Speaker:** Adolfo Correa, M.D.

**Topic:** The Epidemiology of Heart Defects

**Objectives:**

1) Describe geographic and temporal variations in congenital heart defects.
2) Describe one racial/ethnic pattern for congenital heart defects.
3) Identify three known causes of cardiovascular defects.

**Speaker:** Matt Forrester, B.S.

**Topic:** Prevalence and Patterns of Selected Cardiovascular Defects in Texas

**Objectives:**

1) Name the four most common severe cardiovascular birth defects from the Texas Birth Defects Registry.
2) For several selected heart defects, compare Texas rates with rates U.S. or international rates.
3) Identify one significant pattern of heart defects, either by maternal age, place of residence, or maternal education.

**Outline:**

1) Background-the importance of heart defects, risk factors
2) Description (and diagrams) of severe heart defects to be studied in detail
   a) Conotruncal defects
   b) Truncus arteriosus
   c) Transposition of the great arteries
   d) Tetralogy of Fallot
   e) Hypoplastic left heart syndrome
3) Graphs demonstrating the impact of various demographic factors on these defects
   a) Total prevalence
   b) Pregnancy outcome
   c) Region and year of delivery
   d) Border vs. non-border
   e) Race/ethnicity
   f) Maternal age
   g) Sex
   h) Mortality rate
   i) Prenatal diagnosis

**Abstract:** This session examined severe cardiovascular defects. These included conotruncal defects (truncus arteriosus, transposition of the great vessels, and Tetralogy of Fallot) and hypoplastic left heart syndrome. The total prevalence for these defects was examined as well as the impact of a variety of demographic and diagnostic factors - pregnancy outcome, region, delivery year, border vs. non-border residence, maternal race/ethnicity, maternal age, infant sex, mortality, and prenatal diagnosis.

## Prevalence and Patterns of Selected Cardiovascular Defects in Texas

Mathias Forrester
Epidemiologist
Texas Birth Defects Monitoring Division

## Background

- Structural heart defects occur in 5-8/1000 live births
- 25,000-30,000 infants born in United States each year with heart defects (1 out of every 125-150 births)

## Background

- Most common severe birth defect
- >50% of childhood deaths due to birth defects are the result of heart defects

## Risk Factors

- Family history of heart defect
- Advanced maternal age
- Maternal disease (eg, diabetes)
- Exposure to teratogen
- In utero rubella infection
- Chromosome abnormalities
- Most have no risk factors

## Major Cardiac Defects

- Conotruncal defects - anomalies of the outflow tract of the heart
  - Truncus arteriosus - single common arterial trunk instead of separate pulmonary artery and aorta

## Major Cardiac Defects

- Conotruncal defects - anomalies of the outflow tract of the heart
  - Transposition of the great vessels - transposed great arteries such that the pulmonary artery arises from the left ventricle and the aorta arises from the right ventricle

## Major Cardiac Defects

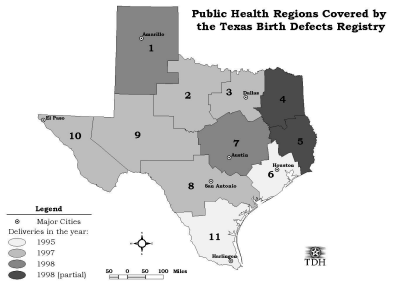
- Conotruncal defects - anomalies of the outflow tract of the heart
  - Tetralogy of Fallot - ventricular septal defect, subvalvular pulmonic stenosis, overriding of the aorta, and right ventricular hypertrophy

## Major Cardiac Defects

- Hypoplastic left heart syndrome - extreme smallness of the left heart structures and aorta (includes hypoplastic left ventricle, mitral valve atresia/hypoplasia, aortic valve atresia/hypoplasia, aorta hypoplasia)

## Texas Birth Defects Monitoring Division (TBDMD)

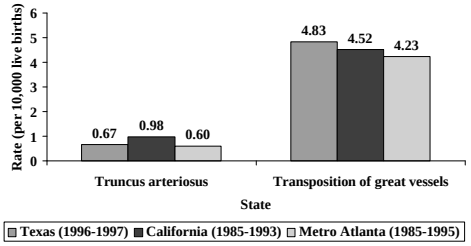
- 1996 - Public health regions 6 and 11
- 1997 - Public health regions 2, 3, 8, 9, 10, & 11
- 300,431 total live births



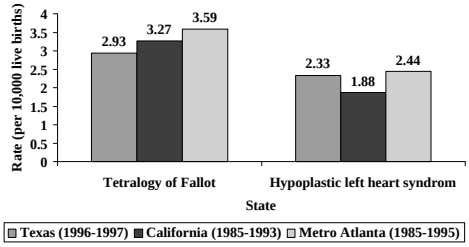

## Texas Birth Defects Monitoring Division (TBDMD)

- 313 total cases
  - 20 Truncus arteriosus
  - 145 Transposition of the great vessels
  - 88 Tetralogy of Fallot
  - 70 Hypoplastic left heart syndrome
  - 10 More than one major cardiac defect

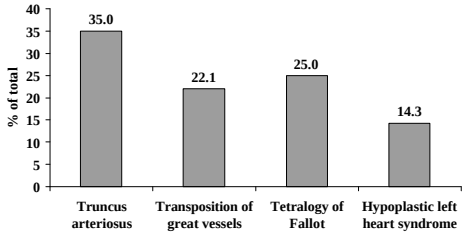
**Prevalence of truncus arteriosus and transposition of the great arteries by state**



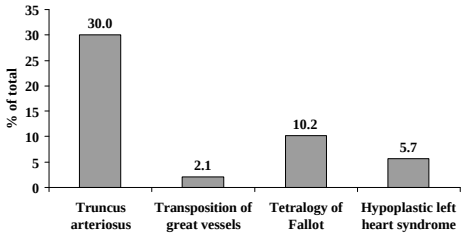
**Prevalence of Tetralogy of Fallot and hypoplastic left heart syndrome by state**



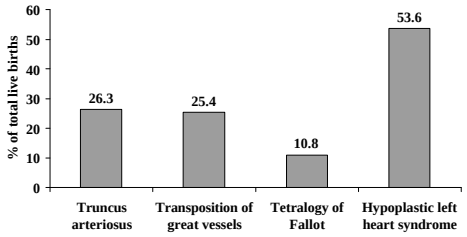

**Proportion of major cardiac defects associated with chromosomal and other syndromes, Texas, 1996-1997**



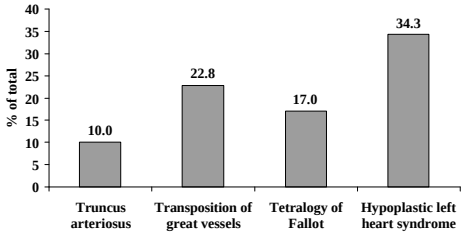

**Proportion of major cardiac defects associated with chromosome 22q deletion, Texas, 1996-1997**



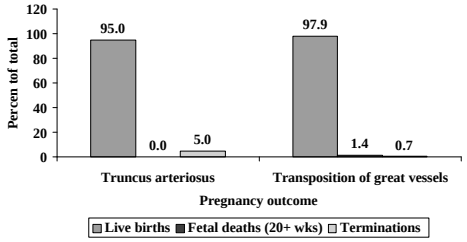

**Proportion of live births with major cardiac defects that expired within one year of age, Texas, 1996-1997**



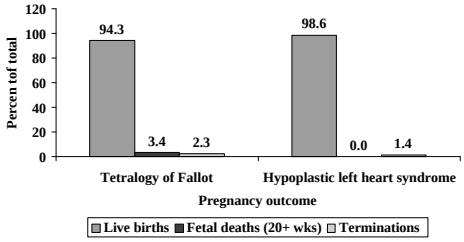

**Proportion of major cardiac defects with prenatal detection of a cardiac defect, Texas, 1996-1997**



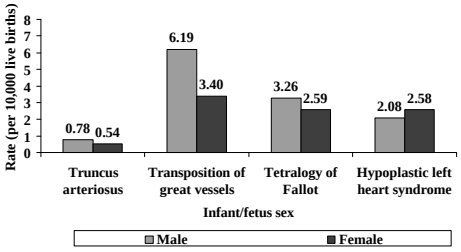

**Distribution of truncus arteriosus and transposition of the great vessels by pregnancy outcome, Texas, 1996-1997**



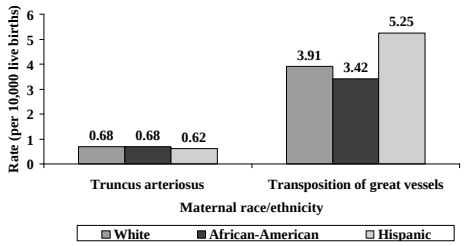
**Distribution of Tetralogy of Fallot and hypoplastic left heart syndrome by pregnancy outcome, Texas, 1996-1997**



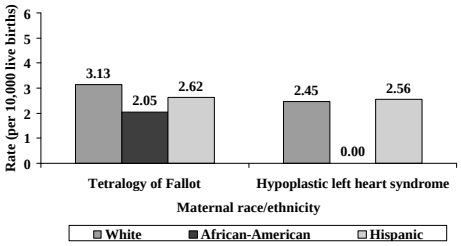

**Prevalence of major cardiac defects by infant/fetus sex, Texas, 1996-1997**



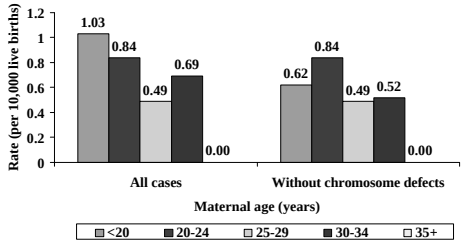

**Prevalence of truncus arteriosus and transposition of the great vessels by maternal race/ethnicity, Texas, 1996-1997**



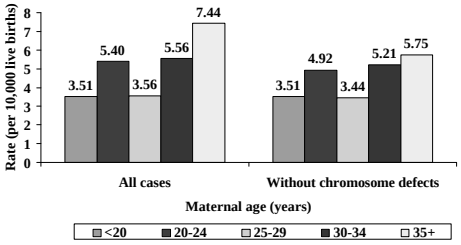

**Prevalence of Tetralogy of Fallot and hypoplastic left heart syndrome by maternal race/ethnicity, Texas, 1996-1997**



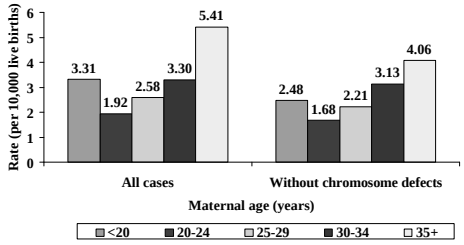

**Prevalence of truncus arteriosus by maternal age, Texas, 1996-1997**



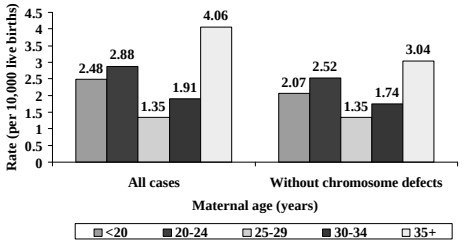

**Prevalence of transposition of the great vessels by maternal age, Texas, 1996-1997**



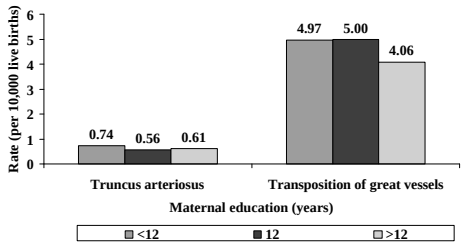
**Prevalence of Tetralogy of Fallot by maternal age, Texas, 1996-1997**



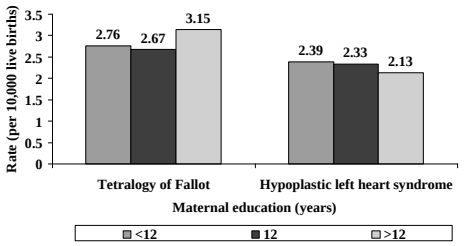

**Prevalence of hypoplastic left heart syndrome by maternal age, Texas, 1996-1997**



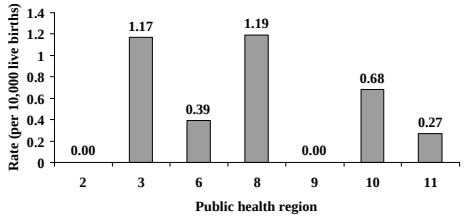

**Prevalence of truncus arteriosus and transposition of the great vessels by maternal education, Texas, 1996-1997**



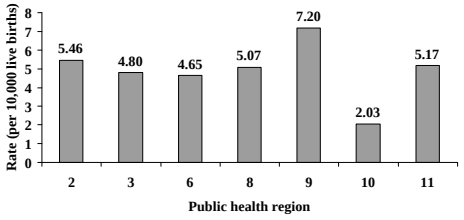

**Prevalence of Tetralogy of Fallot an hypoplastic left heart syndrome by maternal education, Texas, 1996-1997**



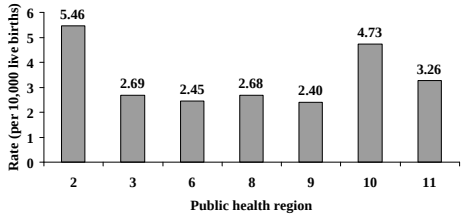

**Prevalence of truncus arteriosus by Public health region, Texas, 1996-1997**



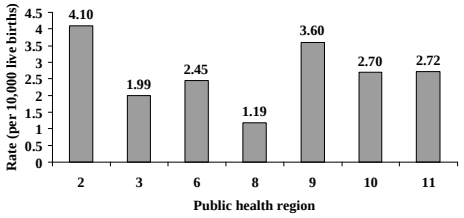

**Prevalence of transposition of the great vessels by Public health region, Texas, 1996-1997**



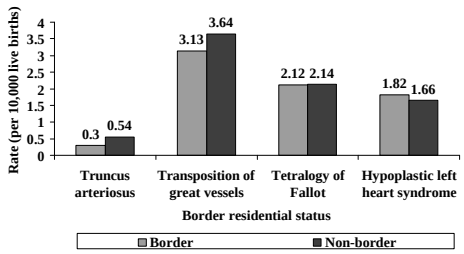
**Prevalence of Tetralogy of Fallot by Public health region, Texas, 1996-1997**




**Prevalence of hypoplastic left heart syndrome by Public health region, Texas, 1996-1997**




**Prevalence of major cardiac defects by border residential status, Texas, 1996-1997**



**Speaker:** John Belmont, M.D., Ph.D.

**Topic:** The Classification and Genetics of Congenital Cardiovascular Defects

**Objectives:**

1) Distinguish between conotruncal defects and outflow-related defects.
2) Define Tetralogy of Fallot
3) Identify the major embryological classes of heart defects and give one example of a genetic cause for each.

**Abstract:** Congenital heart defects (CHD) constitute the single most common anatomic class of birth defects and are a major cause of infant mortality. Correlation of normal and pathological embryology/anatomy has led to the formulation of mechanistic models, but there is limited understanding of the genetic basis for the inferred embryological processes. Most evidence points to extensive etiologic heterogeneity and a reevaluation of simple multifactorial models is required. The recent identification of several genes responsible for congenital heart defects in the context of more complex clinical disorders provide significant entry points for the genetic analysis of human heart development. The association of aneusomies (particularly microdeletion syndromes) with specific cardiac lesions provides further strong support for genetic classification. Studies in the mouse and zebrafish are laying the groundwork for a comprehensive genetic model of cardiac organogenesis. Nevertheless, the basis for the large majority of CHD, especially isolated defects, remains obscure. Dissection of the genetic components of CHD is one of the greatest challenges in medical genetics for the coming decades.

**Speaker:** John Bricker, M.D.

**Topic:** Advances in the Treatment of Heart Defects

**Objectives:**

1) Describe current research on the treatment/repair of hypoplastic left heart
2) Name the treatment which has undergone the most dramatic improvement in last 10-15 years.
3) Discuss prospects for improvements in treatment in the near future.
4) Discuss the current practices and future prospects for *in-utero* surgery of heart defects.

**Outline:**

1) Pharmacologic treatment of babies with congenital cardiac malformations
   a) Prostaglandin
   b) Indomethacin
   c) Afterload reduction
   d) Inotropes
   e) Diuretic therapy
   f) Treatment of pulmonary hypertension
2) Interventional procedures in the cardiac catheterization laboratory
   a) Atrial septostomy
   b) Balloon valvuloplasty
   c) Balloon angioplasty
   d) Stents
   e) Closing the ductus and fistulas
   f) ASD closure
   g) VSD closure
   h) Future developments
3) Surgical treatment of heart defects in childhood
   a) Current strategies for blue heart disease (the AT=s@)
   b) HLHS
   d) Obstructive and regurgitant lesions
   e) Left-to-right shunt lesions
   f) Results and current expectations for the baby with a heart defect
4) Transplantation
   a) Cardiac transplantation
   b) Lung and heart-llung transplants
   c) Xenografts
   d) Future developments
5) Ventricular assist devices and mechanical support
   a) ECMO
   b) LVAD=s
6) Fetal therapy
   a) Arrhythmia management
   b) Fetal surgery and interventions

**Abstract:** Treatment for congenital heart disease was unavailable until the late 1940's. In the second half of the 20th century, advances have occurred which result in treatments being used for almost all types of congenital cardiac malformations. An overview is provided of the various treatments currently in use at major cardiac centers in the United States. Current outcomes of medical and surgical treatments and the expectations for optimal treatment of cardiac disease in childhood will be reviewed. A number of areas for future new therapies will be highlighted as well.

**Speaker:** Richard Finnell, Ph.D.

**Topic:** Genetics of the Folic Acid Pathway: Implications for NTD Prevention

**Objectives:**
1) Name at least one important enzyme of the folic acid pathway that is under genetic control.
2) Describe how genetic differences may impact folic acid levels and the occurrence of neural tube defects.
3) Summarize scientific findings on the genetic aspects of folic acid transport and metabolism.

**Outline:**
1) Name at lease one important enzyme of the folic acid pathway that is under genetic control
2) Describe how genetic differences may impact folic acid levels and the occurrence of NTDs.
3) Summarize scientific findings on genetic aspects of folic acid transport and metabolism

**Abstract:** In the past decade it has become clear that periconceptional folic acid supplementation can significantly reduce both the occurrence and recurrence risks associated with neural tube defects (NTDs). The fact that not all women enjoy the same protective effects from this vitamin supplementation, suggests that underlying genetic factors are involved. Efforts to understand the complex gene-nutrient interactions underlying the development of NTDs have progressed slowly. To date, efforts have focused on genes in the folate biosynthetic pathway that are either involved with folic acid metabolism, or with its transport into target cells. Specifically, the 5,10-methylenetetrahydrofolate reductase gene, which codes for the key enzyme involved in converting potentially toxic homocysteine to methionine, has been suggested to be a risk factor for spina bifida in several populations. In terms of folate transport, the folic acid receptor alpha was considered to be a strong candidate gene for explaining the population burden of NTDs since when this gene is inactivated in a knockout mouse model, the embryos die in utero with NTDs. Subsequent studies of this gene in humans have revealed promising gene conversion events within the coding region that may be associated with increased NTD risk. The role of these genes in NTD pathogenesis will be discussed.

## Why Study Mice to Find Human Genes ?

- 10s of 1000s of ESTs KNOWN FOR BOTH
- 85% HOMOLOGY AT GENOMIC LEVEL
- PROTEINS ARE SIMILAR
- FUNCTIONS ARE SIMILAR
- BIOLOGY IS SIMILAR
- WE SIGN THEIR CONSENT FORMS !

## Genotype versus Phenotype

**GENOTYPE**

Genes Looking for Function

| | |
|---|---|
| Gene Sequencing | Positional Cloning |
| Transgenics | Expression Array |
| Gene Targeting | Mutagenesis |
| Genome Sequence | Proteomics |

**PHENOTYPE**

Function Looking for Genes

## STRATEGY TO IDENTIFY CANDIDATE GENES

IDENTIFY RELEVANT RISK FACTOR

- Low Folic Acid Concentration
- High Homocysteine Concentration

## STRATEGY TO IDENTIFY CANDIDATE GENES

IDENTIFY RELEVANT RISK FACTOR

**SELECT MUTANT WITH RELEVANT PHENOTYPE**

No Available Model with Low Folate and High Homocysteine Concentration
•Develop Folbp1 Knockout Mouse
•Develop RFC Knockout Mouse

## STRATEGY TO IDENTIFY CANDIDATE GENES

IDENTIFY RELEVANT RISK FACTOR

SELECT MUTANT WITH RELEVANT PHENOTYPE

SURVEY PHENOTYPES IN MUTANTS

PERFORM GENETIC ARRAYS TO PROFILE GENE EXPRESSION

## CENTRAL DOGMA

DNA

RNA

PROTEINS

PATHWAYS

SYSTEMS

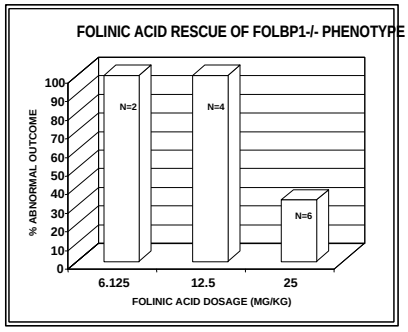
PHENOTYPES

## Consequences of Having an Abnormal Folate Receptor

- Ingested folate is converted to 5-MeTHF
- Each 5-MeTHF molecule is filtered 13 times daily by the kidney
- Renal resorption is dependent upon folate transport
- Defective FR results in lower mean and peak plasma folate concentrations
- Defective maternal FR could decrease placental exposure to 5-MeTHF
- Defective placental FR could decrease fetal 5-MeTHF acquisition

## Folate Receptor Gene Family

| Gene | Formerly Known as/by | Mouse Homolog | Chromosome Location |
|---|---|---|---|
| FRα | MFP2-Lacey et al, 1989 | Folbp-1 | 11q13.3-11q13 |
| FRβ | MFP1-Ratnam et al, 1989 | Folbp-2 | 11q13 |
| FRγ | Shen et al, 1994 | | |
| FRpseudogene | | | 11q13 |
| FRδ | | Folbp-3 | 11q14 |

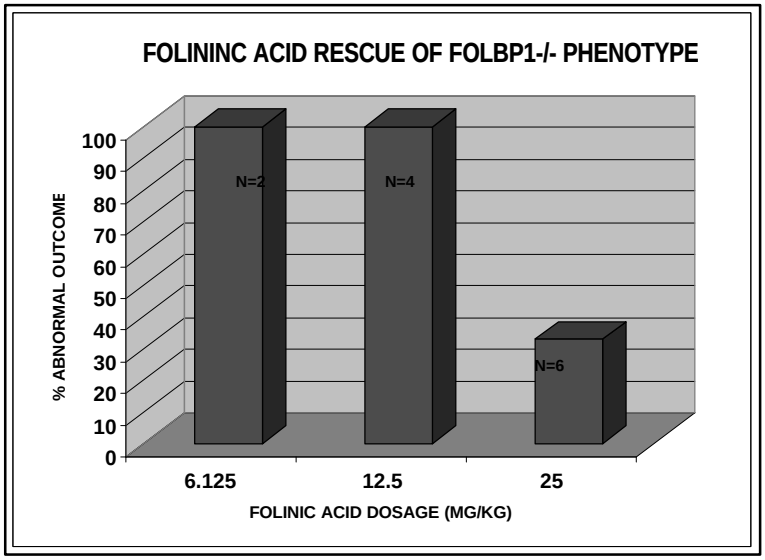


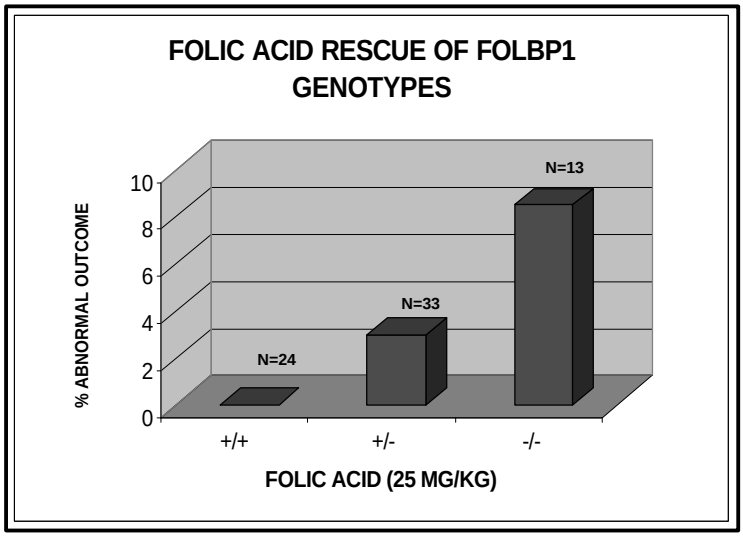

## Five Steps of Receptor Mediated Folate Transport Associated with Craniofacial Malformations/Dysmorphogenesis

- High affinity binding of folate to the receptor
- Translocation of the ligand-receptor complex into the cytoplasm
- Dissociation of folate from the receptor due to proton gradient
- Movement across membrane by an anion-carrier (RFC)
- Polyglutamation of folate in cytoplasm to retain folate within cell

## For Experiments Concerning Genetic Susceptibility to Teratogenesis, the Mouse is the Ideal Organism

- Over 45,000 individual genes have been identified
- Over 40,000 individual genes have been mapped and sequenced
- Over 450 inbred mouse strains exist


FOLININC ACID RESCUE OF FOLBP1-/- PHENOTYPE
% ABNORMAL OUTCOME
100
90
80
70
60
50
40
30
20
10
0
N=2
N=4
N=6
6.125
12.5
25
FOLINIC ACID DOSAGE (MG/KG)


FOLIC ACID RESCUE OF FOLBP1 GENOTYPES
% ABNORMAL OUTCOME
10
8
6
4
2
0
N=24
N=33
N=13
+/+
+/-
-/-
FOLIC ACID (25 MG/KG)

**STRATEGY TO IDENTIFY CANDIDATE GENES**

IDENTIFY RELEVANT RISK FACTOR
SELECT MUTANT WITH RELEVANT PHENOTYPE

**SURVEY PHENOTYPES IN MUTANTS**

•Folbp1 Nullizygotes Die *in Utero* with NTDs, Cardiac and Craniofacial Malformations

•Supplemental Folic Acid Prevents Birth Defects

## Folbp1 Knockout Mice

- Implicates importance of folate transport in early embryogenesis
- Folate deficiency either directly effects target cells by altering function of downstream genes
- Folate deficiency leads to hyperhomocysteinemia which directly affects target cell physiology

## COLLABORATING INVESTIGATORS

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Dr. Leslie Biesecker Dr. William Parvan

California Birth Defects Monitoring Program
Dr. Gary Shaw Dr. Suzann Carmichael

Children's Hospital Oakland
Dr. Ed Lammer

## COLLABORATING INVESTIGATORS

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## Prevalence of Cleft Lip and Palat

- Child with cleft born every two minutes
- 660 children with clefts born each day
- 235,000 children with clefts born each year
- 3,200 more clefts each year worldwide
- Lifetime medical costs exceed $100K in US
- Total lifetime medical costs exceed $750M for single year cohort in US

## Prevalence of Cleft Lip and Palate in US

- One child with a cleft born every 566 newborns
- 7,500 infants born with clefts annually
- Lifetime medical costs exceed $100K
- Total lifetime medical costs exceed $750M for singl year cohort

## Folic Acid and Homocysteine Metabolism

- Linked at branch point of methionine regeneration, transsulfuration and tetrahydrofolate regeneration pathwa
- No direct evidence that homocysteine is a causative age
- Marker of low folate or reduced methionine synthesis
- Mothers of NTD affected infants do not necessarily have elevated homocysteine concentrations

## Closed Dysraphic Malformations

- Not detected by prenatal ultra-sound scanning
- Represents large group of infants with spinal abnormalit
- Includes: spinal lipomas, sacral agenesis, split cord syndromes
- Appear isolated or in association with spina bifida apert

## Why Study Mice to Find Human Genes ?

- **10s of 1000s of ESTs KNOWN FOR BOTH**
- **85% HOMOLOGY AT GENOMIC LEVEL**
- **PROTEINS ARE SIMILAR**
- **FUNCTIONS ARE SIMILAR**
- **BIOLOGY IS SIMILAR**
- **WE SIGN THEIR CONSENT FORMS !**

## Genotype versus Phenotype

**GENOTYPE**

Genes Looking for Function

| | |
|---|---|
| Gene Sequencing | Positional Cloning |
| Transgenics | Expression Array |
| Gene Targeting | Mutagenesis |
| Genome Sequence | Proteomics |

**PHENOTYPE**

Function Looking for Genes

## STRATEGY TO IDENTIFY CANDIDATE GENES

IDENTIFY RELEVANT RISK FACTOR
SELECT MUTANT WITH RELEVANT PHENOTYPE

**SURVEY PHENOTYPES IN MUTANTS**

•Folbp1 Nullizygotes Die *in Utero* with NTDs, Cardiac and Craniofacial Malformations

•Supplemental Folic Acid Prevents Birth Defects

## Evidence Supporting Folate Receptor Genes as Strong Candidates for Regulating Folate Preventable Neural Tube Defects

- **5-MeTHF binding to Folbps is the only known folate specific step in folate transport**
- **Folbps regulate intracellular folate concentrations**
- **Folbps are localized to few tissues, all epithelialized, including:kidney, choroid plexus, placenta and developing neuroepithelium**

## Generating Folbp Knockout Mice

- **Isolate genes from 129Sv genomic DNA library**
- **Targeting construct creates deletion as well as insertional inactivation in exon 2 of Folbp1**
- **Targeting constructs introduced into ES cells**
- **Colonies screened by selective media and positives tested by multiple Southern Blots**
- **Germ line chimeras generated**
- **Establish knockout lines**

## Human Polymorphism Detection Studies

- **Do polymorphisms exist in the gene of interest in the human population ?**
- **If so, determine if there is a positive relationship between selected forms of the gene and an increased risk for neural tube defects**

## Gene Conversion in Folate Receptor α

- **Chimeric exon 7 has 3 missense mutations- M233L, F244L, and S247N**
- **Patient with A7394G produced a K198R amino acid substitution**
- **Both M233L and K198R mutations involve residues that are highly conserved among all FR isoforms**
- **All mutations affect the GPI anchor region of the protein**

## Folate Receptor Gene Family

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